TITLE: Altered Apolipoprotein C expression in association with cognition impairments and hippocampus volume in schizophrenia and bipolar disorder

Abbreviated Title: Apolipoprotein alterations in psychosis

Authorship

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Abstract

Proteomic analyses facilitate the interpretation of molecular biomarker probes which are very helpful in diagnosing schizophrenia. In the current study, we attempt to test whether potential differences in plasma protein expressions in schizophrenia (SZ) and bipolar disorder (BD) are associated with cognitive deficits and their underlying brain structures.

42 plasma proteins of 29 SZ patients, 25 BD patients and 93 non-clinical controls were quantified and analysed using multiple reaction monitoring (MRM) based triple quadrupole mass spectrometry approach. We also computed group comparisons of protein expressions between patients and controls, and between SZ and BD patients, as well. Potential associations of protein levels with cognitive functioning (psychomotor speed, executive functioning, crystallized intelligence) as well as underlying brain volume in the hippocampus were explored, using bivariate correlation analyses.

The main finding of this study was that apolipoprotein (ApoC) expression differed between patients and controls; and that these alterations in both disease groups were putatively related to cognitive impairments as well as to hippocampus volumes. However, none of the protein level differences were related to clinical symptom severity.

In summary, altered apolipoprotein expression in BD and SZ was linked to cognitive decline and underlying morphological changes in both disorders. Our results suggest that the detection of molecular patterns in association with cognitive performance and its underlying brain morphology is of great importance for understanding of the pathological mechanisms of SZ and BD, as well as for supporting the diagnosis and treatment of both disorders.

1. Introduction

Diagnosis of schizophrenia (SZ) or bipolar disorder (BD) is normally based on clinical interviews of patients by psychiatrists. However, conventional symptom-based descriptive models of bipolar disorder (BD) and schizophrenia (SZ) have recently been complemented by a neurobiological approach. According to this biological approach not only do both disorders share some clinical features or cognitive impairments, but they also share biological mechanisms underlying the clinical and cognitive features. Biological mechanisms of interest are assessed by functional (fMRI) and structural neuroimaging (sMRI) data, or protein expressions (proteomics). In general, previous proteomic studies in mental disorders yielded changes in metabolic and immunological pathophysiological pathways, partly independently of treatment or illness status (Schwarz et al., 2010; Schwarz et al., 2012). Moreover, mass spectrometry-based proteomics have identified proteins which may serve as biomarker candidates to prognosis, diagnosis, and medication monitoring in peripheral tissue (Nascimento & Martins-de-Souza, 2015).

However, whether changes in the levels of serum proteins may be directly correlated with cognitive or clinical features of psychiatric disorders has rarely been investigated. A major purpose of the study is to assess whether protein expressions reflect psychiatric illnesses such as BD or SZ. A further aim is to link potential pathophysiological abnormalities of BD and SZ with cognitive impairments and their underlying brain structure. Apart from clinical features, schizophrenia (SZ) and bipolar disorder (BD) share common alterations in cognitive domains, e.g., episodic or working memory (Mann-Wrobel, Carreno, & Dickinson, 2013; Schaefer, Giangrande, Weinberger, & Dickinson, 2013). These observations challenge previous dichotomous approaches of psychosis (Kraepelin, 1919) and indicate the need for searching biological (e.g., neuroimaging, molecular) markers underlying shared symptoms of both disorders.

Specifically, Apolipoprotein (Apo) levels has been identified as risk factor for several neuropsychiatric diseases (Elliott, Weickert, & Garner, 2010), e.g. for Alzheimer's disease. Additionally, previous evidence supports the hypothesis that changes in Apo concentrations may be involved in the psychopathology of SZ (Lee, Kim, & Song, 2013; La et al., 2007; Wu et al., 2013; (Dassati, Waldner, & Schweigreiter, 2014; Dean, Digney, Sundram, Thomas, & Scarr, 2008; Takahashi et al., 2008) (Dean et al., 2008; Digney, Keriakous, Scarr, Thomas, & Dean, 2005; Martins-De-Souza et al., 2010; Sasaki, Funakoshi, & Arakawa, 1985) and BD (Dassati et al., 2014). In a recent review of Martins-de-Souza and colleagues, they compared proteomics in serum of 55 first-onset drug-naïve SZ patients and 33 controls and observed, amongst others, the apolipoproteins A1, A2, A4, C1 and D had been differentially expressed. According to the authors, this finding supports the idea that there is, in fact, a phospholipid dysfunction in SZ (Martins-de-Souza, Guest, Rahmoune, & Bahn, 2012).

Furthermore, it has been proposed that altered Apo levels might play a putative contributory role in decreased cognitive performance (Dassati et al., 2014; Elliott et al., 2010; Huang & Mahley, 2014; La et al., 2007). This assumption could be validated for dementia (Kim et al., 2014). However, this link between cognitive impairments and Apo levels has not been done for SZ or BD, so far. A study by Verbrugghe and colleagues conducted and replicated a genetic association analysis between disease outcome and cognitive performance in SZ (Verbrugghe et al., 2012). They reported that ApoE, ApoER2, very-low-density-lipoprotein receptor (VLDLR) and disabled-1 (DAB1) single nucleotide polymorphisms (SNPs) were significantly associated with disease outcome, pre-morbid intelligence and verbal memory. Thus, the authors concluded that neurodevelopmental/synaptic plasticity genes are involved in cognitive deficits in SZ. Nonetheless, to our knowledge, a potential overlap between protein concentrations and clinical or cognitive symptoms in BD and SZ has not been systematically

investigated, yet. Moreover, alterations in cortical and subcortical regions (i.e., basal ganglia, thalamus, cingulate, insula, hippocampus) have been observed (see for review (Ellison-Wright & Bullmore, 2010; Gupta et al., 2015; Shenton, Dickey, Frumin, & McCarley, 2001)) supporting the idea of shared the neurobiological underpinnings of cognitive impairments in both disorders (Ehrlich et al., 2011; Gutierrez-Galve et al., 2011; Hartberg et al., 2010; Hartberg et al., 2011; Knöchel et al., 2016; Knochel et al., 2014; Oertel-Knöchel et al., 2012).

The main purpose of this explorative study is to identify protein levels reflecting the pathomechanisms of BD or SZ in a trans-diagnostic approach in contrast to healthy controls. Another aim is to explore whether changes in levels of proteins are disorder specific or rather reflecting some other phenotype within the patients. It is hypothesised that SZ and BD are the result of partly similar underlying biological factors which could explain the aforementioned clinical similarities. Finally, it is examine whether protein levels are associated with neurocognitive changes and brain structures related to neurocognitive deficits in BD and SZ.

2. Methods

2.1 Participants

We assessed 29 SZ patients [mean age (M_{age}) = 37.16 years and standard deviation (SD) = 10.96 years] and 25 euthymic BD type I patients (M_{age} = 37.79 and SD = 10.40 years) without any comorbid axis I or II disorders (including drug abuse) according to the DSM-IV criteria (APA, 1994). All patients were outpatients of the Department of Psychiatry, Psychosomatic Medicine and Psychotherapy, Goethe-University, Frankfurt, Germany. 93 non-clinical participants (CON; M_{age} = 33.59 and SD = 11.18 years) were also included, using sample search matched criteria. The selection of the controls was done by searching for matching pairs with the SZ and BD patient groups regarding age, gender and education status. Exclusion criteria for

all participants were current drug abuse or any acute infection. Potential group differences in age, gender and years of education were tested for, which revealed no differences across controls, BD or SZ patients in these variables (all *p*-values > 0.05) (see Table 1 for further details). The body mass index (BMI; kg/m^2) based on weight and height was calculated for all participants. Furthermore, in order to confirm diagnosis and rule out (comorbid) psychological and personality disorders in SZ or BD patients, and ruling out any past or present mental illness in the control group, the Structured Clinical Interview for DSM-IV (SCID-I and SCID-II; German version (Wittchen, Zaudig, & Fydrich, 1997)) was conducted with all participants. Only those controls not reporting any mental illness in the past or present and without any first-degree relatives with any mental disease were included into the study. Participants were provided with a description of the study and gave written informed consent before participating. Experimental procedures were approved by the ethical board of the medical department of the Goethe-University, Frankfurt/Main, Germany.

----------Insert Table 1 about here----------

2.2 Data acquisition & preprocessing

Blood sample collection and plasma digestion

Venous blood (10 ml) of all participants was collected in the morning (8am) to avoid circadian interference in biomarker measurements and aliquoted into two 9.0 ml serum tubes (Sarstedt, Numbrecht, Germany). All participants did not eat, drink, or smoke after 10 pm the night before blood sampling until the collection was done. This was ensured using an in-house questionnaire. Furthermore, all participants received detailed information prior to the blood draw.

The blood samples were prepared according to standard protocols (see *e.g.* (de Witte et al., 2014)). Samples were centrifuged at 4800 rpm for 10 minutes to remove clotted cells and other debris. The plasma was then transferred into Geiner Bio-One Reaction Tubes (1.5 ml, PP, graduate, attached cap) and stored at – 80°C.

Plasma proteins were reduced with 10 mM dithiothreitol (DTT) for 30 min at 60 °C and then alkylated with 10mM iodoacetamide (IAA) for 30 min at room temperature in the dark. Tryptic digestion was performed using porcine trypsin (Sequencing Grade Modified, Promega, Winconsin) for 17 hours at 37 °C (Levin, Schwarz, Wang, Leweke, & Bahn, 2007). Samples were then spiked with a mixture of heavy isotope-labeled peptide standards and analyzed by nano Liquid chromatography- Multiple Reaction Monitoring Mass Spectrometry (nano LC–MRM-MS).

Mass spectrometry based protein quantification

Tryptic digested proteins were quantified using Waters Xevo Triple quadrupole coupled to a nanoAcquity UPLC system (Waters Corporation, UK) and operated in the multiple reaction monitoring (MRM) mode as described previously (Wesseling, Gottschalk, & Bahn, 2014). The LC buffer system was as follows: mobile phase A, 0.1% formic acid and mobile phase B, 0.1% formic acid in acetonitrile. The peptides were separated and eluted at a flow rate of 300 nL/min using a 48-minute gradient of 97/3% (A/B) to 60/40% in 30 minutes; 60/40% to 15/85% in 2 minutes; 5 minutes at 15/85%; and returning to the initial condition in 1 minute. The trapping and analytical columns were C18 (180 µmx20mm, 5-µm particle size) and C18 BEH nano-column (75 µmx200mm, 1.7-mm particle size), respectively.

Forty-two plasma proteins were quantified using Psynova Human Blood Panel-42 (Psynova Neurotech Ltd. Cambridge, UK)(Alsaif et al., 2012; Enaw & Smith, 2013; Schwarz et al., 2012; Schwarz et al., 2010a)(see Table 2). This selection was based on current findings in Schizophrenia and Bipolar disorder regarding the relevance of plasma proteins for etiological models of the diseases (see e.g., (Alsaif et al., 2012; Enaw et al., 2013; Schwarz et al., 2012; Schwarz et al., 2010b)).

----------Insert Table 2 about here----------

Briefly, unique peptides and fragment ions for each targeted proteins were selected as described previously (Wesseling et al., 2014). The peptides were then quantified along with spiked stable-isotope-labeled internal standards of the same sequence. Each sample was analysed in triplicate.

Assessment of cognitive and clinical data

Before conducting the MRI measurements, all participants were screened regarding their clinical symptomatology by an independent clinical expert. Furthermore, all participants underwent a psychometric assessment by an investigator of the study.

Prior to clinical ratings, all participants completed the German version of the "Spot-the-Word-Test" (MWT-B) to measure *crystallised intelligence* (Mehrfachwahl-Wortschatz-Test) (Lehrl, 2005) and the Trail Making Test A (TMT A) for *psychomotor speed* as well as the TMT B for *executive functioning* (Reitan, Hom, & Wolfson, 1988) (see Table 1 for further details).

To guarantee homogeneity between patient groups, it was ensured that the duration of illness (at a minimum of five years), the onset of disease and years of drug treatment were comparable between SZ and BD patients (see Table 1 for further details). All the patients' treatment regimens were stable during the month preceding testing. There were no medication-free patients; also none of them had received benzodiazepine drugs in the precedent month. All SZ patients were treated with second generation antipsychotic medications (in some cases also with first generation antipsychotics) at time of assessment. BD patients' medication was grouped into three categories: lithium (lithium, lithium + antidepressant), other mood stabilisers (other mood stabilisers, other mood stabilisers + antidepressant) and antipsychotics (neuroleptics, neuroleptics + antidepressant) (see Table 1). In order to compare different substances and doses, chlorpromazine equivalents concerning antipsychotics (see the formula by (Woods, 2003)), amitryptiline equivalents concerning antidepressant drugs (Ali, 1998), and

mg of lithium, valproic acid and lamotrigine were computed. Furthermore, a 'medication load' based on a method first introduced by Almeida (Almeida et al., 2009) was calculated.

Depressive symptom severity was assessed using the Beck Depression Inventory (BDI II) (Hautzinger, Keller, & Kühner, 2006), a widely accepted scale in Germany for the assessment of acute depressive symptoms. BD patients had a mean of 10.21 (9.39) in the BDI II sum scores indicateing subclinical depressive symptoms in BD patients, whereas controls had a mean of 2.09 (7.76) points in BDI II. Mania symptoms in BD patients and controls were measured using the widely used Bech-Rafaelsen Mania Scale (BRMAS) (Bech, 1981). Both, BD patients and controls had non-clinical scores of the BRMAS (BD patients: *M*=2.10 (2.75), controls: *M*=1.45 (1.12). However, none of the patients or controls had a score >19 in the BDI II and a score >7 in the BRMAS, which would indicate a clinically relevant symptomatology during testing (see Table 1).

In addition to that, SZ patients were also tested for potential acute symptoms using the Positive and Negative Syndrome Scale (PANSS) (Kay, Fiszbein, & Opler, 1987). The following mean PANSS scores were present in SZ patients: global scale: *M*=66.85 (8.34), positive symptoms: *M*=17.01 (5.34), negative symptoms: *M*=17.88 (5.87) and general symptomatology: *M*=33.01 (8.49).

Anatomical measurement

Within one week of the data assessment, each participant underwent a high-resolution T1-weighted anatomical measurement (MDEFT sequence; (Deichmann, Schwarzbauer, & Turner, 2004), 176 slices, 1x1x1 mm) using a Siemens Magnetom Allegra 3 Tesla MRI system (Siemens Medical Systems, Erlangen, Germany) at the Goethe University Brain Imaging Centre, Frankfurt, Germany. Participants were scanned under dimmed lights and instructed to lie still and look at a white fixation cross presented in the centre of the visual field. Participants did not engage in any overt speech during the scanning sequences. The anatomical MRI scans

of all participants were reviewed by a neuroradiologist who did not find any clinically relevant pathology.

A voxel-based morphometry approach (VBM) was applied to preprocess and analyse the anatomical data, using the SPM8 running on MATLAB® version 7.7.0 (Statistical Parametric Mapping [Wellcome Department of Imaging Neuroscience, London, UK]). All images were checked for artefacts, structural abnormalities and pathologies by two independent raters. Next, customized T1 templates and prior images of grey matter (GM), white matter (WM) and cerebro-spinal fluid (CSF) were created from all participants for use in the group analysis. We used modulated data and prior probability maps (voxel intensity) to guide segmentation. The segmentation included six different tissue types, light bias regularisation (0.001), 60 mm bias FWHM cut-off, warping regularisation of 4 mm, affine regularisation to the ICBM European brain template (linear registration) and a sampling distance of 3 mm. The quality of the segmentation was checked before further analysis. Finally, the images were smoothed with a Gaussian kernel of 8 x 8 x 8 mm (FWHM). Using this procedure, the intensity of each voxel was replaced by the weighted average of the surrounding voxels, blurring the segmented image.

The pre-processed data were analysed using a region-of-interest (ROI) approach. Therefore, we took anatomical defined masks of the left and right hippocampus (see Figure 1 for an illustration and detailed information of the masks) from the WFU PickAtlas toolbox in SPM8 (Maldjian, Laurienti, Burdette, & Kraft, 2003). The left hippocampus mask included 929 volumes, the right hippocampus mask 949 volumes. The relative volume of each participant (t-statistic for each voxel), using intracranial volume (ICV) as a nuisance variable, in the left and right hippocampus were extracted using the Marsbar-Tool® implemented in the SPM software. The resulting relative volume were extracted and imported into an SPSS 22.0 data sheet for further statistical testing.

----------Insert Figure 1 about here----------

2.3 Statistical analysis

The statistical methods of this study were reviewed by the Department of Biostatistics, Faculty of Medicine, Goethe-University Frankfurt/Main, Germany. The strategy regarding statistical analyses included three steps.

First, for MRM analysis of the protein data, raw data of protein concentrations were processed using Skyline software and analysed using the MSstats® package (Choi et al., 2014) in R software (Core Team, 2014). This resulted in normalised log transformed relative intensities of protein concentrations, which were exported into SPSS® (SPSS, 2014) to test group comparisons. Group comparisons showed a statistical trend (p=0.09) regarding BMI scores due to lower scores in the control group in comparison with SZ and BD patients. BMI was a confounder [that is, BMI was associated with both protein levels and group ($p < 0.05$)] in some models. Consequently, to control for BMI, it was included in all statistical comparisons. Using the SPSS 22.0 software, linear mixed models (linear; random intercept) were performed on the protein levels, computing the following factors:

1. Comparisons of protein levels between patients (SZ and BD patients) and controls
2. Comparisons of protein levels between BD and SZ patients

Significance was based upon a threshold p-value defined using Bonferroni and an α-level of 0.05.

Second, bivariate correlation analyses using Pearson product-moment or Spearman Rank correlation coefficients (Bonferroni corrected) were performed to examine the relationships between the protein concentrations and other variables of interest (cognitive scores, hippocampus volume). However, only protein concentrations revealing significant contrasts during group comparisons of patients versus controls (first level) were included in these analyses. We also controlled for the potential influence of medication by performing bivariate correlation analyses (Spearman product-moment correlation, two-tailed) between the

protein scores and the medication doses, as well as the duration of medication in patients. Finally, we controlled for potential influence of clinical variables (BDI II, BRMAS, PANSS) on the protein concentrations, computing additional bivariate correlation analysis (Spearman rank correlation).

3. Results

3.1 Group comparisons of plasma protein concentration (MRM)

Contrasts between patients (SZ and BD patients) and controls: Protein expressions in patients versus controls

The following proteins showed different expressions in patients compared with controls: ANT3, ApoA1, ApoA2, ApoA4, ApoC1, ApoC2, ApoC3, ApoC4, ApoL1, C1QC, CFAB, CO3, F13B, FCN3, HEP2, HRG, KLKB1, PEDF, RET4 (all $p_{Bonf} < 0.05$).

Contrasts between disease groups: Protein expression in BD versus SZ patients

The following proteins showed significant dissimilarities between BD and SZ patients: A2AP, ANT3, ApoB, ApoD and ApoF (all $p_{Bonf} < 0.05$). In comparison to SZ patients, BD patients displayed the tendency of having higher concentrations in the aforementioned proteins (see Table 3).

----------Insert Table 3 about here----------

----------Insert Figure 1 about here----------

3.2 Correlation analyses

In SZ patients, the negative association between ApoC2 scores and psychomotor speed (TMT A) and right HC volumes reached a significant level. In line with that, ApoC3 scores were inversely correlated with left and right hippocampus volumes, psychomotor speed and executive functioning. We also detected significant negative correlations between ApoC4

scores and volumes of the right HC and psychomotor speed. ApoL1 scores were positively correlated with right hippocampus (all $p \leq 0.05$; Bonferroni corrected; see Figure 3, Table 4 for details).

In BD, higher crystallised intelligence scores were significantly associated with lower ApoC3 and ApoC4 levels, whereas bilateral HC volumes were inversely associated with ApoC3 values and executive functioning was as well negatively correlated with ApoC3 levels (all $p \leq 0.05$). No significant relationship between the protein levels and any of the clinical scores across groups could be observed (all $p > 0.05$).

Computing bivariate correlation analyses independently of diagnosis groups (all participants included), lead to results comparable to those found when doing the analyses for each group individually: significant correlations between APOC2, APOC3 and APOL1 and HC right volume; significant correlations between APOC2 and APOC4 with TMT A; and significant correlations between APOC3 and TMT B as well as HC left volume could be observed (please see Table 4 for further details).

----------Insert Figure 3 about here----------

The differences between the subject groups regarding gender, age and education did not reach statistical significance. Therefore, this variable was not included into the main analysis. However, additional correlation analyses were computed between the main results (ApoC values and age, gender as well as education) for each subject group individually. These analyses demonstrate no significant influence of age, gender and education to the protein values ($p > 0.05$).

We controlled for potential association between affective symptom severity in BD patients and controls (BDI II, BRMAS) and cognitive performance (MWT-B, TMT A, TMT B) and could not found any significant relationship ($p > 0.05$). Accordingly, additional correlation analyses between cognitive performance (MWT-B, TMT A, TMT B) and psychotic symptoms

in SZ patients were conducted, in order to control for potential influence of symptom severity on the cognitive performance. However, no significant association between symptoms of psychosis as measured with the PANSS and cognitive performance in the cognitive domains measured with the TMT A, TMT B or MWT-B could be found ($p > 0.05$).

3.6 Medication effects

In SZ patients, high chlorpromazine equivalent scores were correlated with high ApoA4 levels ($p = 0.414$, $rho = 0.044$). ApoA4 levels also correlated significantly with Almeida scores in BD patients ($p = 0.453$, $rho = 0.045$). None of the other protein concentrations revealed any significant association with medication scores. Duration of medication (in years) was not correlated with any of the protein levels (all $p < 0.05$).

----------Insert Table 4 about here----------

4. Discussion

Having outlined the results of the study at hand, the major findings will discussed in the following. First of all, a number of protein levels that differing between patients (SZ and BD) and controls could be observed. Secondly, A2AP, ApoB, ApoD, ApoF and ANT3 displayed differences across patient groups (SZ vs. BD). Moreover, apolipoprotein (ApoC) expressions were not only different between patients and controls, but were putatively related to cognitive impairments, as well as to hippocampus volumes. However, none of the protein level differences were related to clinical symptom severity.

Recent disease models of psychosis suggest that there are shared dysfunctional biological pathways contributing to the clinical signs of both disorders (Consortium et al., 2009; O'Donnell, Vohs, Hetrick, Carroll, & Shekhar, 2004; Sanchez-Morla et al., 2008; Smith, Barch, & Csernansky, 2009) but that there are also distinct symptoms and pathomechanisms. Changes in Apo concentrations have been demonstrated by several authors and have been linked to the

physiopathology of SZ (Lee, Kim, & Song, 2013La et al., 2007; Wu et al., 2013)(Sasaki et al., 1985; Takahashi et al., 2008). The involvement of apolipoprotein dysfunction in the pathophysiology of SZ was also suggested by Martins-de-Souza and colleagues (Martins-de-Souza et al., 2012). Accordingly, the results of the study at hand are consistent with those of Haenisch and colleagues (Haenisch et al., 2014) who conducted a multiplex analysis of plasma protein concentrations and described 26 dysregulated proteins in BD, including growth factors, hormones, lipid transport (*e.g.* APOA1) and inflammatory proteins. Our results are independent of acute symptomatology, although our study was neither powered nor specifically designed to assess such correlations since our patient samples showed relatively low severity scores for acute symptoms.

However, as a main outcome of the current study, direct associations between cognitive impairments, underlying structural abnormalities and altered protein expression in apolipoproteins could be observed in both disease groups. Primarily, we detected significant inverse correlations between concentrations of ApoC2, ApoC3, ApoC4 and psychomotor speed in SZ. As well, poorer executive functioning was inversely correlated with ApoC3 concentration alterations in SZ and BD patients, both. This was accompanied by significant inverse correlations between ApoC3 and ApoC4 levels and crystallised intelligence in BD; *i.e.* the poorer the cognitive performance, the higher the changes in Apo concentrations. Apo are involved in the metabolism and transport of lipids within the CNS as well as functioning in brain physiology. The involvement of altered Apo levels in decreased cognitive performance or cognitive decline has been proposed previously (Dassati et al., 2014; Elliott et al., 2010; Huang & Mahley, 2014; La et al., 2007), but has not yet been thoroughly investigated in SZ or BD. A study by Verbrugghe et al. (Verbrugghe et al., 2012) conducted an association analysis between disease outcome and cognitive performance in SZ. They described that ApoE, ApoER2, VLDLR, and DAB1 SNPs were significantly related to disease outcome, pre-morbid

intelligence and verbal memory. Thus, it was concluded that neurodevelopmental/synaptic plasticity genes are involved in cognitive deficits in SZ.

Secondly, bilateral hippocampal (HC) volume was also inversely related to levels of ApoC3 in BD and SZ, *i.e.* the lower the volume, the higher the changes in Apo concentrations. Furthermore, ApoC2, Apo4 and ApoL1 levels were associated with right HC volumes in SZ patients. As the hippocampus plays a central role in cognitive functioning (Avery, Williams, Woolard, & Heckers, 2013) this finding may be of great importance. Taking into account that SZ patients display more cognitive deficits than BD patients, a stronger connection between imaging markers related to the HC and Apo concentrations may indicate a reflection of those deficits in SZ patients. Our results are in line with the report by Hwang and colleagues (Hwang et al., 2013), who detected that abnormal immune/inflammation response in the hippocampus was associated with abnormalities in the parvalbumin-containing neurons. The authors proposed that this altered hippocampus response might lead to the cognitive deficits underlying the pathophysiology of SZ. Consistenly, Vila-Rodriguez and co-workers (Vila-Rodriguez, Honer, Innis, Wellington, & Beasley, 2011) observed a significant negative association between ApoE concentration and GM volumes. Moreover, (Serra-Grabulosa et al., 2003) did not only state that ApoE and ApoC1 polymorphisms are genetic risk factors for dementia and cognitive impairment in the elderly, they also found that ApoC1 is associated with changes in hippocampal volumes in elderly people with cognitive impairments. Although those results were not corrected for sociodemographic differences, they hint towards a connection between ApoC1, neuronal changes in the area of the HC and cognitive deficits.

Strength and limitations

Although in this study, it was carefully controlled for confounders, such as acute symptomatology or medication dosage, these variables might still influence the direction and intensity of protein changes. The relationship between metabolic problems, such as obesity,

diabetes and elevated lipids, and the expression of Apo has been frequently investigated in the general population (Padrao, Ferreira, Vitorino, & Amado, 2012; Ramachandran et al., 2012; Sadler et al., 2012). Due to antipsychotic medications in SZ and mood stabilisers in BD, those patients have an increased risk for metabolic disturbances and altered lipid metabolism (Gibbons, Thomas, Scarr, & Dean, 2010), ultimately contributing to an increased risk for cardiovascular disease (Meyer et al., 2008). Several authors observed an association between Apo concentrations in patients receiving psychiatric medication (e.g., phenothiazines, olanzapine, risperidone) (Dean et al., 2008; Sasaki et al., 1985; Smith, Segman, Golcer-Dubner, Pavlov, & Lerer, 2008; Song et al., 2014). Therefore, we controlled for potential effects of the duration of drug treatment, the dosage of the current treatment, as well as BMI values on the protein concentrations. No relationship between psychiatric medication use, BMI, and any of the protein concentrations except for ApoA4 concentration could be found (which was a parameter that did not differ significantly across groups). Nevertheless, in order to control for potential metabolic side effects, in the future a group of patients not taking any psychiatric medication might be helpful to detect potential impacts of medication on the findings.

Another limitation of this current study could be seen in its relatively small size. However, this can be attributed to the very strict inclusion criteria; the severity of symptoms were comparable; ensuring that none of the patients had any history of drug addiction; and participant groups were carefully matched. Consequently, taking into account the inherent lack of reproducibility in proteomic studies (Song et al., 2014), the results need to be replicated in larger samples. Hence, further investigations regarding multiple protein concentrations in a single study may be necessary to detect potential links between clinical outcome or cognitive decline and the markers of interest.

Conclusion

Regarding the discussion about shared or distinct pathophysiological pathways in BD and SZ (see (Kraepelin, 1896) and (Craddock & Owen, 2005)), we present evidence indicating that both disorders share predominant Apo alterations which are connected to psychomotor speed, executive functioning and crystallised intelligence, as well as to hippocampus volume. Therefore, this result indicates that Apo might be useful biomarkers for cognitive dysfunction in both disorders, consistent with the NIMH RDoC framework (Wang & Insel, 2010).

Furthermore, the strong correlation of cognitive performance and volume in HC with Apo levels in SZ could be a reflection of the latter suffering from more severe deficits. Accordingly, based on the fact that none of the assessed clinical parameters were associated with serum levels of protein markers, it could be assumed that the protein levels are associated with cognitive deficits rather than with affective or psychotic symptoms in BD and SZ.

Intriguingly, our results are consistent with recent genome-wide association studies revealing partial, but not complete, overlap of the genetic risk profiles for BD and SZ (Hall et al., 2014). Our results suggest that the detection of molecular patterns in association with cognitive performance. Its underlying brain morphology is of great importance in the understanding of the pathological mechanisms of SZ and BD and may lead to novel biological diagnostic tools (Haenisch et al., 2014). Although the presented findings are of great interest to psychiatric research, further studies are needed to explore and explain these graded changes in SZ and BD in order to define which parameters show graded changes and which do not. The changes in the Apo plasma level before and after treatment with antipsychotics should also be further investigated. Finally, as present findings in other disorders, e.g., Alzheimer's disease, and the fact that both, SZ and BD display overlapping results regarding apolipoprotein dysfunctions, it could suggested/ assumed that these changes are not specific for psychosis but instead rather characterise a phenotype across psychiatric disorders related to cognitive functioning.

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Table 1: Sociodemographic, clinical and cognitive variables across groups.

Mean and SD (in brackets). BDI II = Beck Depression Inventory, PANSS = Positive and Negative Syndrome Scale, f = female, m = male. SZ = schizophrenia, BD = bipolar patients, CON = controls. * = significant differences on α level of $p \leq 0.05$; ** = significant differences on α level of $p \leq 0.01$; *** = significant differences on an α level of $p \leq 0.001$.

	SZ	**BD**	**CON**	**Statistics p**
Number	29	25	93	-
Gender (f / m)	8 / 21	6 / 19	39 / 44	Kruskal-wallis: *p*=0.156
Age (years) M (SD)	37.16 (10.96)	37.79 (10.40)	33.59 (11.18)	*p* = 0.117
Education (years) M (SD)	15.09 (3.19)	15.19 (2.37)	16.14 (1.96)	*p*=0.06
Handedness	all right handed			
Duration of illness (years) M (SD)	11.81 (7.79)	8.860 (5.47)	-	*p* = 0.06
Onset of disease (years) M (SD)	25.75 (5.34)	29.60 (10.98)	-	*p* = 0.07
BMI (kg/m^2) M (SD)	28.33 (6.37)	28.12 (3.23)	24.09 (15.33)	*p* = 0.09
Medication (years) M (SD)	6.88 (4.56)	7.23 (2.34)	-	*p*=0.37
Medication equivalents M (SD)	Chlorpromazine equivalents (mg/day): 580.92 (230.12)	Medication load: 2.44 (1.19) Amitriptyline-equivalent (mg/day): 123.12 (80.23) Valproic acid (mg/day): 1215.14 (801.53)		
Medication categories	please see Table S2	please see Table S2	-	-
BDI II	10.85 (3.13)	10.21 (9.39)	2.09 (7.76)	*p* = 0.0009***
BRMAS	-	2.10 (2.75)	1.45 (1.12)	*p*=0.70
PANSS	PANSS pos: 17.01 (5.34), PANSS neg: 17.88 (5.87)			

	PANSS gen: 33.01 (8.49), PANSS sum: 66.85 (8.34)			
Medication categories	quetiapin (n=25) quetiapin + antidepressant (n=1) risperidon + antidepressant (n=1) amisulprid + antidepressant (n=1) apipriprazol + antidepressant (n=1) quetiapin + haloperidol (n = 5) apipriprazol + haloperidol (n = 4)	lithium (n = 13) lithium + bupropion (n=1) lithium + valdoxan (n=1) lamotrigin (n=3) valproin acid (n=1) lamotrigin + antidepressant (n = 1) quetiapin (n=4) quetiapin + antidepressant (n=1)		

Table 2: Complete list of the assessed serum markers according to the immunoassay platforms *Human ImmunoMAP*® and *Human DiscoveryMAP*® of the MyriadRBM®. Abbreviations = Abbv. Sources: * = uniprot.org, ** = rbm.myriad, a = (Gagliardi, Ho, & Torpy, 2010), b = (Kashuba, Bailey, Allsup, & Cawkwell, 2013).

Number	Serum protein	Abbv.	Function
	MRM-assessment		
1	**Alpha 1-antitrypsin**	A1AT	acute-phase-protein*
2	**Alpha 2-antiplasmin/ plasmin inhibitor**	A2AP	thrombogene*
3	**Alpha 2-macroglobulin**	A2MG	thrombogene*
4	**Adenine nucleotide translocase 3**	ANT3	apoptosis*
5	**Apolipoprotein A1**	APOA1	lipid transport*
6	**Apolipoprotein A2**	APOA2	lipid transport*
7	**Apolipoprotein A4**	APOA4	lipid transport*
8	**Apolipoprotein B**	APOB	lipid transport*
9	**Apolipoprotein C1**	APOC1	lipid transport*
10	**Apolipoprotein C2**	APOC2	lipid transport*
11	**Apolipoprotein C3**	APOC3	lipid transport*
12	**Apolipoprotein C4**	APOC4	lipid transport*
13	**Apolipoprotein D**	APOD	lipid transport*
14	**Apolipoprotein E**	APOE	lipid transport*
15	**Apolipoprotein F**	APOF	lipid transport*
16	**Apolipoprotein H**	APOH	lipid transport*
17	**Apolipoprotein L1**	APOL1	lipid transport*
18	**Apolipoprotein M**	APOM	lipid transport*
19	**Apolipoprotein O**	APOO	lipid transport*
20	**Transcortin**	CBG	regulation of cortisol activity[a]
21	**Ceruloplasmin**	CERU	acute-phase-protein*
22	**Clusterin**	CLUS	apoptose, complement induced celllyse *
23	**Complement component 3**	CO3	acute-phase-protein, complement system *
24	**Complement C4A**	CO4A	complement system*
25	**Complement component C9**	CO9	complement-system*
26	**Complement C1q**	C1QC	complement system*
27	**Complement factor B**	CFAB	complement system*
28	**Ficolin-3**	FCN3	complement system*
29	**Coagulation factor XIII B/ clotting factor**	F13B	koagulation, fibrin stabiliser*
30	**HSP70 Escort Protein 2**	HEP2	apoptose*
31	**Haptoglobin**	HPT	acute-phase-protein*
32	**Haptoglobin related protein**	HPTR	acute-phase-protein*

33	**Histidine-rich glycoprotein**	HRG	immunological*
34	**C1-inhibitor/ C1 esterase inhibitor**	IC1	complement system*
35	**Immunoglobulin heavy constant μ**	IGHM	immunological*
36	**Plasma-Kallikrein**	KLKB1	Koagulation, Kallikrein-Kinin system[b]
37	**Matrix metalloproteinase 9**	MMP9	immunological *
38	**Pigment epithelium-derived factor**	PEDF	immunological *
39	**Phosphatidylinositol-Glycan-specific phospholipase D**	PHLD	lipid metabolism *
40	**Plasminogen**	PLMN	immunological, acute-phase-protein, fibrinlyse*
41	**Retinol-binding protein 4**	RET4	sugar metabolism**
42	**Transferrin**	TRFE	immunological**

Table 3: Mean and Standard Deviations across groups and Statistical group comparisons. The data are normalised log transformed relative intensities of protein concentrations. We used linear mixed models (linear; random intercept) of the protein data adjusted for BMI scores. SZ = schizophrenia, BD=bipolar patients, CON =controls, PAT=patients. Mean = mean, SD = standard deviation. * = significant differences on α level of $p \leq 0.05$; ** = significant differences on α level of $p \leq 0.01$; *** = significant differences on an α level of $p \leq 0.001$.

Scores	**SZ** *M* (*SD*)	**BD** *M* (*SD*)	**PAT** *M (SD)*	**CON** *M* (*SD*)	**Statistical Group Comparison patients vs. controls**	**Statistical Group Comparisons** BD / SZ *p*
A2AP	17.495 (0.819)	18.013 (0.447)	17.717 (0.496)	17.571 (0.709)	F=0.582, p=0.563	0.009**
ANT3	17.425 (1.112)	18.196 (0.524)	17.791 (1.033)	17.460 (0.993)	F=4.143, p=0.024*	0.011*
APOA1	21.963 (0.231)	23.015 (0.132)	22.511 (1.504)	22.430 (0.123)	F=3.684, p=0.034*	0.253
APOA2	17.963 (1.769)	18.858 (0.307)	18.476 (1.350)	18.186 (1.125)	F=3.655, p=0.035*	0.247
APOA4	20.097 (0.123)	20.411 (0.l43)	20.307 (0.698)	20.123 (1.023)	F=3.887, p=0.029*	0.155
APOB	15.997 (0.264)	16.119 (0.379)	16.054 (1.114)	16.207 (0.396)	F=0.368, p=0.694	0.005**
APOC1	19.367 (1.039)	20.096 (0.326)	19.470 (0.958)	19.130 (0.966)	F=6.127, p=0.005**	0.278
APOC2	21.938 (0.759)	21.882 (0.653)	21.896 (0.715)	21.501 (0.572)	F=6.338, p=0.004**	0.207
APOC3	22.441 (0.965)	22.704 (0.653)	22.518 (0.809)	22.209 (0.526)	F=6.902, p=0.003**	0.261
APOC4	18.982 (0.404)	18.834 (0.543)	18.905 (0.416)	18.819 (0.327)	F=4.828, p=0.014*	0.172
APOD	19.597 (0.181)	19.850 (0.261)	19.683 (0.669)	19.657 (0.272)	F=0.728, p=0.489	0.050*
APOE	19.428 (0.202)	19.465 (0.291)	19.435 (0.889)	19.312 (0.303)	F=2.705, p=0.080	0.077
APOF	17.990 (0.164)	18.129 (0.137)	17.950 (0.644)	17.992 (0.236)	F=0.470, p=0.629	0.015*
APOL1	18.079 (0.669)	18.199 (0.251)	18.111 (0.572)	19.009 (0.377)	F=5.269, p=0.010*	0.214
C1QC	16.343 (0.342)	16.224 (0.125)	18.711 (0.794)	16.376 (0.125)	F=3.845, p=0.030*	0.167
CFAB	19.582 (0.519)	19.609 (0.225)	19.599 (0.503)	19.401 (0.473)	F=6.580, p=0.004**	0.278
CO3	22.790 (0.686)	22.673 (0.185)	22.703 (0.537)	22.652 (0.302)	F=4.931, p=0.012*	0.221
F13B	17.222 (0.123)	17.177 (0.423)	17.190 (0.517)	17.327 (0.195)	F=3.938, p=0.028*	0.175

FCN3	19.581 (0.776)	19.634 (0.418)	19.602 (0.616)	19.548 (0.463)	F=5.055, p=0.011*	0.187
HEP2	18.857 (0.927)	19.290 (0.718)	19.090 (0.800)	18.863 (0.594)	F=6.109, p=0.005**	0.330
HRG	19.324 (0.596)	19.332 (0.362)	19.309 (0.501)	19.454 (0.343)	F=4.594, p=0.016*	0.172
KLKB1	18.495 (0.461)	18.252 (0.203)	18.310 (0.388)	18.483 (0.264)	F=4.057, p=0.025*	0.266
MMP9	17.994 (0.187)	17.668 (0.123)	17.787 (0.496)	17.583 (0.128)	F=3.079, p=0.058	0.160
PEDF	19.294 (0.866)	19.453 (0.370)	19.401 (0.662)	19.277 (0.608)	F=4.853, p=0.013*	0.209
RET4	19.917 (0.234)	20.001 (0.154)	19.989 (0.587)	19.856 (0.768)	F=3.922, p=0.028*	0.148

Table 4: Significant correlations (two-tailed; Bonferroni corrected). Spearman rank correlation = rho, Pearson Product Moment correlation = r, SZ = schizophrenia patients, BD = bipolar patients, CON = controls. TMT A = Trail Making Test A, MWT-B = Mehrfachwahl-Wortschatz-Test (Spot-the-Word-Test), HC= hippocampus volume.

	Significant correlations		
Protein	**BD**	**SZ**	**Total**
APOC2		TMT A: *r*=-0.45, *p*=0.002 HC right: *r*=-0.54, *p*=0.006	TMT A: *r*=-0.35, *p*=0.001 HC right: *r*=-0.25, *p*=0.02
APOC3	MWT-B: *r*= -0.56, *p*=0.009 HC left: *r*=-0.62, *p*= 0.003 HC right: *r*=-0.65, *p*= 0.002 TMT B: *r*=-0.49, *p*=0.008	HC left: *r*=-0.45, *p*=0.003 HC right: *r*= -0.41, *p*=0.004 TMT A: *r*=-0.52, *p* = 0.01 TMT B: *r*=-0.43, *p* = 0.01	HC left: *r*=-0.39, *p*=0.03 HC right: *r*=-0.26, *p*=0.01 TMT B: *r*=-0.48, *p*=0.01
APOC4	MWT-B: *r*=-0.56, *p*= 0.009	TMT A: *r*=-0.49, *p* = 0.01 HC right: *r* =-0.51, p= 0.01	TMT A: *r*=-0.37, *p*=0.001
APOL1		HC right: *r*=0.42, *p*=0.004	HC right: *r*=0.25, *p*=0.02
APOA4	Almeida: *p* = 0.453, *rho* = 0.045*	Chlorpromazine: *p* = 0.414, *rho* = 0.044*	

Figure 1: Illustration of the masks used for the VBM analysis. The masks were extracted using the WFU PickAtlas toolbox in SPM8 (Maldjian et al., 2003) and the Marsbar-Tool. Hippocampus mask left: -28, -15, -25 (929 volumes); hippocampus mask right: 21, -21, -24 (949 volumes).

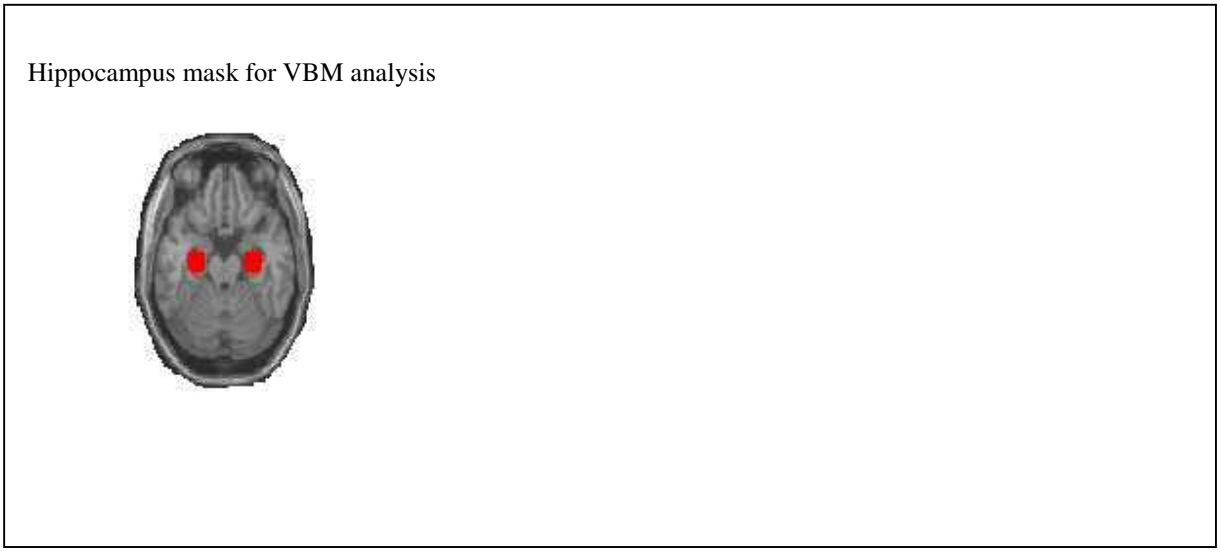
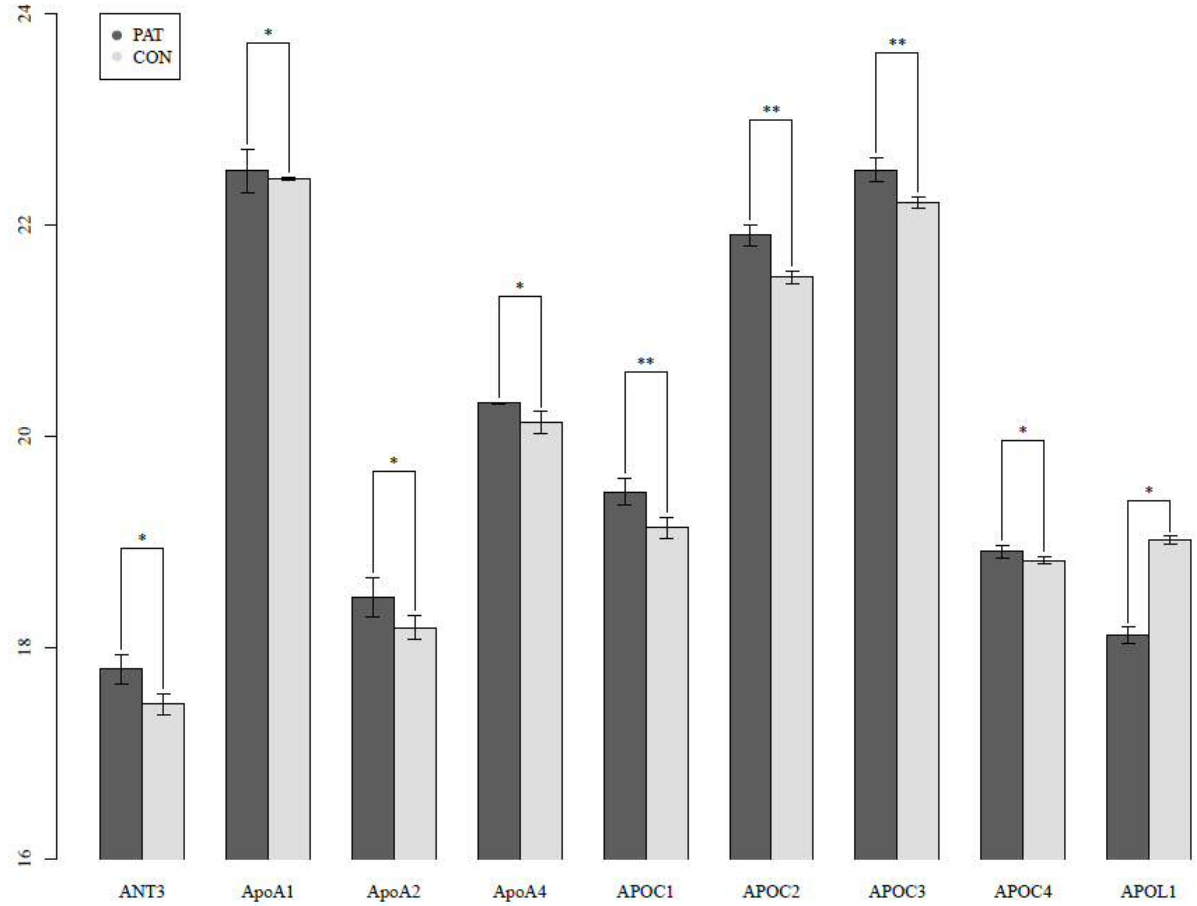


Figure 2: Illustration of the statistical group comparisons, using linear mixed models (linear; random intercept) of the protein data adjusted for BMI scores. We show those results which deemed significant during group comparisons. A. shows all significant contrasts between patient groups (BD and SZ) and controls, B. shows all significant contrasts between BD and SZ patients. Abbreviations: SZ = schizophrenia, BD = bipolar patients, CON = controls. Mean = mean, SD = standard deviation.

A)


PAT
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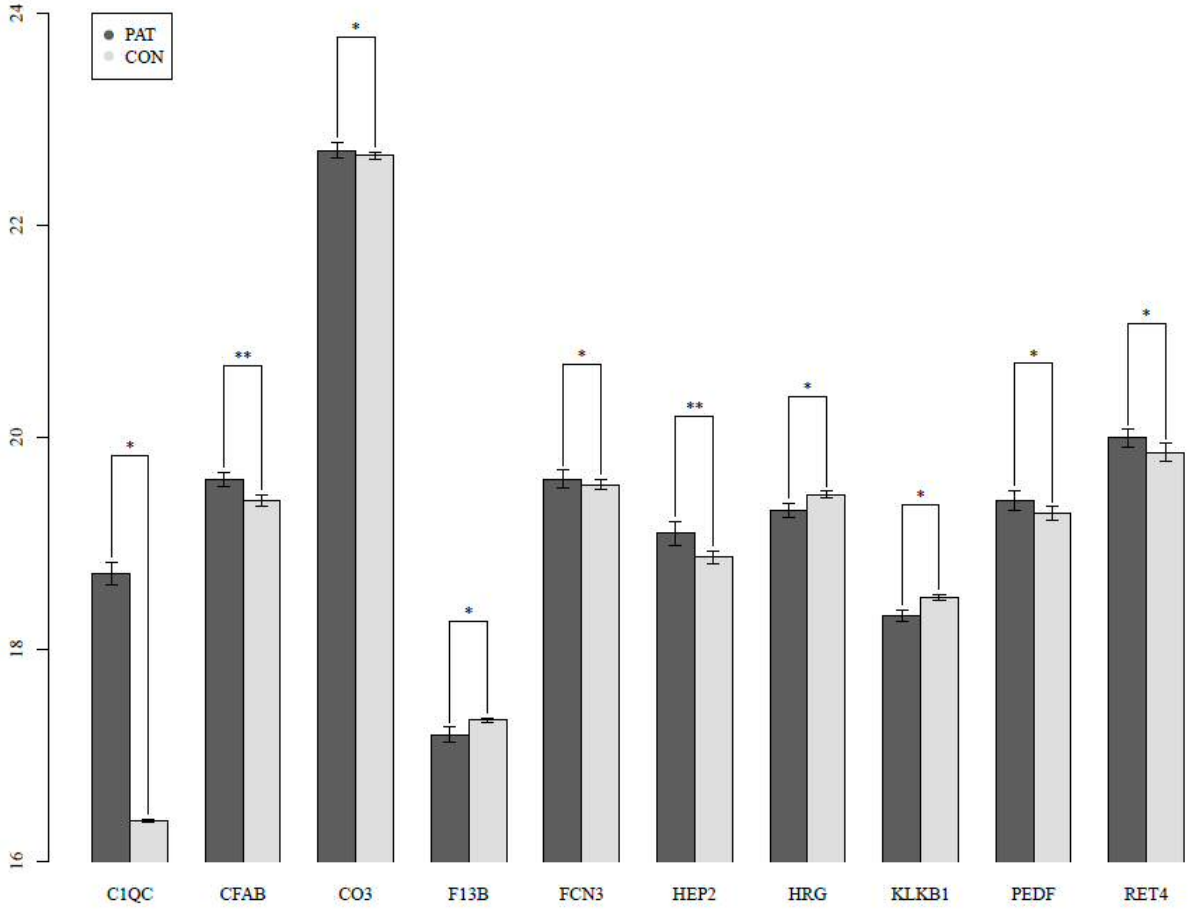
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KLKB1
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RET4

B)

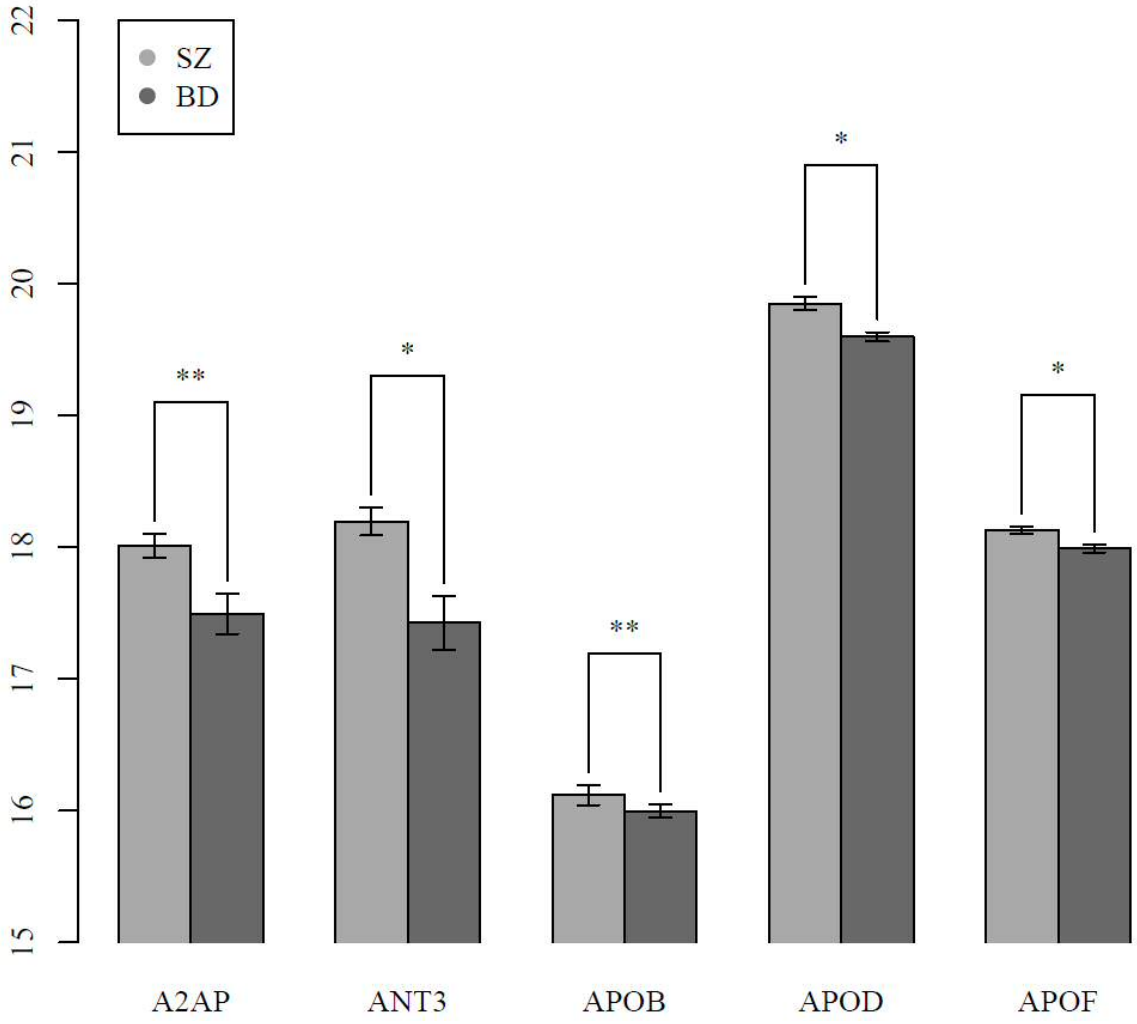

Figure 3: Illustration of the main findings of the study and the functional relevance of the protein alterations.